GREEN SYNTHESIS OF NANOPARTICLES USING *Pongamia pinnata* LEAF EXTRACT

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

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During the course of the project, she exhibited dedication, scientific aptitude, and a keen interest in research. The work submitted is original and meets the academic standards of the institute.

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "Green Synthesis of Iron Oxide Nanoparticles using *Pongamia pinnata* Leaf Extract" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology is an authentic record of my own work carried out during the period from January, 2025 to April, 2025 under the supervision of Dr. Chitrangada Das Mukhopadhyay, Center of Healthcare Science and Technology, Indian Institute of Engineering Science and Technology (IIEST), Shibpur.

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Plagiarism Self-Declaration

I Mahvish Arzoo, hereby declare that the project work titled " Green Synthesis of Iron Oxide Nanoparticles using *Pongamia pinnata* Leaf Extract " presented for the partial fulfilment of the requirements for the Master of Science degree to the Department of Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata.

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ABBREVIATION

1. PVA	Polyvinyl Alcohol
2. IONP	Iron Oxide Nanoparticles
3. XRD	X-Ray Diffraction
4. FTIR	Fourier Transform Infrared Spectroscopy
5. ZVINP	Zero Valent Iron Nanoparticle
6. MG	Malachite Green
7. Fe-P-NP	Iron Polyphenol Complex
8. MCB	Mono-chloro-benzene
9. IV	Intravenous
10. MPS	Mononuclear Phagocyte System
11. RES	Reticuloendothelial System
12. GC-MS	Gas Chromatography-Mass Spectrometry
13. SPR	Surface Plasmon Resonance

ABSTRACT

The green synthesis of nanoparticles and their application in drug delivery have garnered significant attention as a sustainable and efficient alternative to conventional chemical methods. This innovative approach leverages the biocompatibility of nanocarriers, minimizing the toxicity associated with hazardous chemicals and enhancing their suitability for biomedical applications. Recent research has extensively explored various nanoparticle-based drug delivery systems to improve the efficacy and safety of drug.

In this study, we synthesized iron oxide nanoparticles using environmentally friendly biological sources and evaluated their anti-microbial potential. Iron Oxide is a mineral compound that presents main forms such as hematite, magnetite, and maghemite (Cornell and Schwertmann 2000; Zboril et al. 2002; Teja and Koh 2009; Babay et al. 2005). The synthesized nanoparticles were initially characterized using UV-Vis spectroscopy and further analysed through Fourier Transform Infrared Spectroscopy (FTIR), Dynamic Light Scattering (DLS) techniques. Additionally, their surface charge properties were assessed via Zeta Potential analysis and cell viability analysis was performed on HeLa cell lines.

The result section addresses the data of the characterization methods involved to test the formation of the nanoparticle by the UV Visible Absorbance obtained between 200 – 800 nm, DLS also known as Photon Correlation Spectroscopy analysis was done to know the size and size distribution of the particle in liquid suspension. XRD reading suggested the lack of well-defined crystalline structure with a broad hump. FTIR spectroscopy was done to analyse the functional group present on the nanoparticles.

Keywords: Iron Oxide Nanoparticles, Green Synthesis, Biomedical Application, Nanocarriers, Sustainable Nanotechnology

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1. INTRODUCTION

Nanotechnology is an emerging field of study with a great extent of application, from antimicrobial to drug vehicle applications. Nanotechnology operates in a world where matter is controlled at the atomic and molecular scale. Nanotechnology is an interdisciplinary field of study concerned with the design,, production, and application of structures, devices, and systems by manipulating matter at the nanoscale within the range of 1 to 100nm, this size is important because at this scale the material exhibits novel physical, chemical, and biological properties which can be exploited in different field of application such as biomedicine, catalysis, environmental application, drug delivery, Huge number of processes can be used for physico-chemical synthesis of nanoparticles involving use of toxic chemicals. There are mainly four types of nanotechnology based on the process and the environment they work;

• Top down: Reduction of material from larger structure nanometer scale
• Bottom down: Structures and materials are built from individual atoms and molecules, assembling the components on the nanometer scale to form larger, more complex structures.
• Dry: The manipulation and fabrication of nanometer materials and structures in a dry environment, where liquids are not present.
• Wet: The manipulation and fabrication of nanometric materials and structures in the presence of liquids, such as water or other solvents.

1.1. Introduction to Nanoparticles

Nanoparticles are the particles that ranges from 1-100 nm in size exhibits a remarkable and interesting properties with their small sizes, large surface area with freely suspended bonds and high reactivity when collated to their bulk alternative (Kubik and Sugisaka 2002; Daniel and Astruc 2004; Zharov et al. 2005). The nanoparticles can be classified into inorganic based, organic based, carbon based or composite based. The nanoparticles are synthesised in different ways which may enhance their physio-chemical properties using methods such as chemical precipitation, sol-gel and forced hydrolysis techniques, hydrothermal techniques, surfactant/template mediated synthesis, biometric mineralisation, precipitation by anhydrous solution, microemulsion techniques, flow injection synthesis, electrochemical methods, aerosol/vapour methods and sono-chemical techniques.[10]. However, the green diminution of metallic precursors in order to create the nanoparticles (NPs) in

cheaper, more environmentally friendly, and toxic chemical-contamination-free forms for use in biomedical and medical applications is the primary objective of this research.
The metallic nanoparticle ranges from 10-100 nm in size. The three different polymeric forms of Iron Oxide are compounds occurring are maghemite, magnetite and wustite. In the nanoscale, these becomes ideal for their surface functionalization and modification for various applications including drug delivery.[9] Iron oxide nanoparticles (IONPs) have garnered attention in recent years due their unique physiochemical properties.

1.2. Green Synthesis of Iron Oxide nanoparticles

$FeCl_3$ quickly releases Fe^{+3} in water, facilitating faster reaction rate in aqueous solution, which reacts with OH- and reduces to form iron oxide in presence of phytochemicals in the plant extract. Ferric chloride ($FeCl_3$) is a hygroscopic salt with high water solubility, making it a suitable option for large-scale nanoparticle synthesis. Fe^{3+} is the key precursor which get reduced to form IONPs synthesis. Phytochemicals have electron donating functional group which acts as a reducing agent to form Fe_2O_3 or Fe_3O_4 containing both Fe^{+3} or Fe^{+2}.

$$FeCl_3 \rightarrow Fe^{3+} + 3Cl^-$$

$$Fe^{3+} + \text{phytochemicals (e.g., polyphenols)} \rightarrow Fe_2O_3 \text{ or } Fe_3O_4 + \text{by-products}$$

Green synthesis utilises biological sources as reducing and capping agent minimising use of conventional toxic chemical methods making the particle suitable for biomedical application. This approach minimises the harmful impact and cost-effective alternative to traditional methods. In this research we utilised the use of plant leaf from *Pongamia pinnata*. The method is termed as ‘green’ as it does not generate polluting byproducts and does not require much infrastructure, reagents or energy expenditure for nanoparticle synthesis. In a meta-analysis, it is found that IONPs generated by the “Green Synthesis” method had three times more pronounced bacteriostatic activity than those generated by coprecipitation method.

1.3. Medicinal Importance of *Pongamia pinnata*

Pongamia pinnata is an outbreeding diploid medium sized, evergreen, perennial and deciduous legume tree in the pea family, Fabaceae, with a diploid chromosome number of 22[4]. It is the only

species in genus *Pongamia* [1]. It is a fast-growing tree and reaches about 40 feet in height and spread, forming a broad, spreading canopy casting moderate shade. It can grow in saline soils, tolerate drought, and help in nitrogen fixation, making it useful for land reclamation and afforestation projects.

The common name of this plant includes Indian beech, Karanja, and Pongame oil tree[2][3]. Their seeds generally contain oil (27-39%), protein (17-37%), starch (6-7%), crude fiber (5-7%), moisture (15-20%), and ash content (2-3%). Oleic acid accounts up nearly fifty percent of the oil in P. pinnata seeds. [5]

Pongamia oil, which is extracted from the seeds, has an elevated level of triglycerides. The bitter flavonoid components, such as karanjin, pongamol, tannin, and karanja chromene, are the root of their terrible smell and flavor. [6] If taken in their purest form, these chemicals can cause nausea and vomiting. The fruits, sprouts and seeds are used in traditional medicine [6]. Its leaves contain a rich array of bioactive compounds using flavonoids (e.g., pongamol, karanjin), alkaloids, tannins, phenolic acids and glycosides (C M NOORJAHAN, 2019). It has been applied as crude drug for the treatment of tumors, piles, skin diseases (Rout at al.,2009; Pavithra et al.,2010). In Ayurvedic medicinal system, they have been effectively employed as wound healer.

Table 1: Taxonomical Classification of *Pongamia pinnata*

TAXANOMIC RANK	**TAXON**
Kingdom	Plantae
Subkingdom	Tracheophytes
Super-division	Angiosperm
Division	Eudicots
Class	Rosids
Order	Fabales
Family	Fabaceae
Subfamily	Faboideae
Tribe	Millettieae
Genus	*Pongamia*
Species	*P. pinnata*

Figure 1 Pongamia pinnata plant at IIEST, Shibpur campus

1.3. Characterisation of Nanoparticles

The characterization analysis of the nanoparticles includes Ultraviolet visible spectroscopy, X-Ray diffraction, Fourier Transform Infrared spectroscopy, Dynamic light scattering and Zeta size analysis.

- **Ultraviolet visible spectroscopy:** An analytical technique used to measure the absorbance and transmittance of UV and visible light by a sample based on the principle that different molecule absorbs specific wavelength. It is based on Beer-Lambert law. It is a valuable tool for characterising nanoparticle and provides information on their stability. The SPR peak decreases in case the aggregation takes place. Iron oxides are the semiconductor or insulator, thus does not exhibit free electrons compared to noble metals like gold or silver.
- **X-Ray diffraction:** It helps in determining crystalline structure of the nanoparticle by directing X-ray over the sample and obtaining the diffraction pattern. When the X-ray interacts with regularly spaced atoms, it scatters in all direction at specific angles. The scattered waves will be in phase and results in constructive interface and the formation of diffracted beam.
- **Fourier Transform Infrared spectroscopy:** This technique provides an insightful information on the chemical bonds and functional groups by analysing its infrared

absorption spectrum. An effective approach for revealing functional groups and chemical bonds in synthesized substances is Fourier Transform Infrared (FTIR) spectroscopy. FTIR is vital for both characterizing the final iron oxide structure and verifying the presence of organic molecules used in the synthesis process when iron oxide nanoparticles are synthesised using green route, where plant extracts or other biological agents are used as reducing and stabilizing agents.

- Dynamic light scattering, referred to as Photon Correlation Spectroscopy (PCS), is a popular method to assess the hydrodynamic particle size distribution and polydispersity index (PDI) of nanoparticles that that are suspended in a liquid medium. DLS offers vital information about the particle size, aggregation behaviour, and colloidal stability of iron oxide nanoparticles made using environmentally friendly processes. These details are crucial for evaluating the caliber and suitability of the produced nanomaterials. The dispersion and stability of the nanoparticles are enhanced by green production methods that use biological or plant extracts as reducing and capping agents to affect the surface charge and inhibit aggregation. Using the Stokes-Einstein equation, DLS estimates the Brownian motion of these nanoparticles in solution and connects it with particle size.

1.4. Application of IRON OXIDE nanoparticles

The IONPs has varied number of applications in the field of biomedicine which includes anticancer, antimicrobial, and treatments on human bone marrow cell and molecular cell binding. IONPs are widely used in medical and biotechnological fields such as- MRI contrasts, cell separation, tissue repair, hyperthermia, and drug delivery due to their unique super-paramagnetism, small size and ability to get coated with biocompatible materials. The synthesized iron oxide nanoparticles (IONPs) are biocompatible, FDA-approved, and exhibit physical and chemical stability, making them safe for biomedical applications. IONPs are also used as MRI contrast agents, this system is very important in the visualisation of bioevents, such as gene expression, and metastasis at cellular and sub cellular levels.

Iron oxide nanoparticles (IONPs) are classified as 'nanozymes' because they possess intrinsic enzyme-mimicking activities and catalytic properties similar to various oxidases, including peroxidase, catalase, sulfite oxidase, and superoxide dismutase. [16]

In cancer hyperthermia, tumour cells are killed by magnetically induced heat. Ferromagnetic materials (like Fe_2O_3, Fe_3O_4) possess hysteretic properties under a time-varying magnetic field,

which induces heat that conducts into the immediately surrounding diseased tissue whereby, if the temperature is maintained above the therapeutic threshold (42°C) for 30 min or more, the cancer is destroyed. Experimental investigations of the application of magnetic materials for hyperthermia was carried out as early as 1957 by Gilchrist et al. [23]

1.5. Bioaccumulation and Biodistribution of Iron oxide Nanoparticles

Iron is a vital trace element found in the human body, playing a central role in various physiological processes, including oxygen transport, DNA synthesis, and cellular respiration. It is a key component of haemoglobin in RBCs, thus help in oxygen transport. Iron is an essential component of the mitochondrial cytochrome and has roles in energy production and cellular respiration. It also exists in myoglobin, a protein found in muscles that accumulates and expends oxygen. Through supporting the growth and development of immune cells, cognitive development, and the generation of neurotransmitters such as dopamine and serotonin, it also plays a vital role in immunological support.

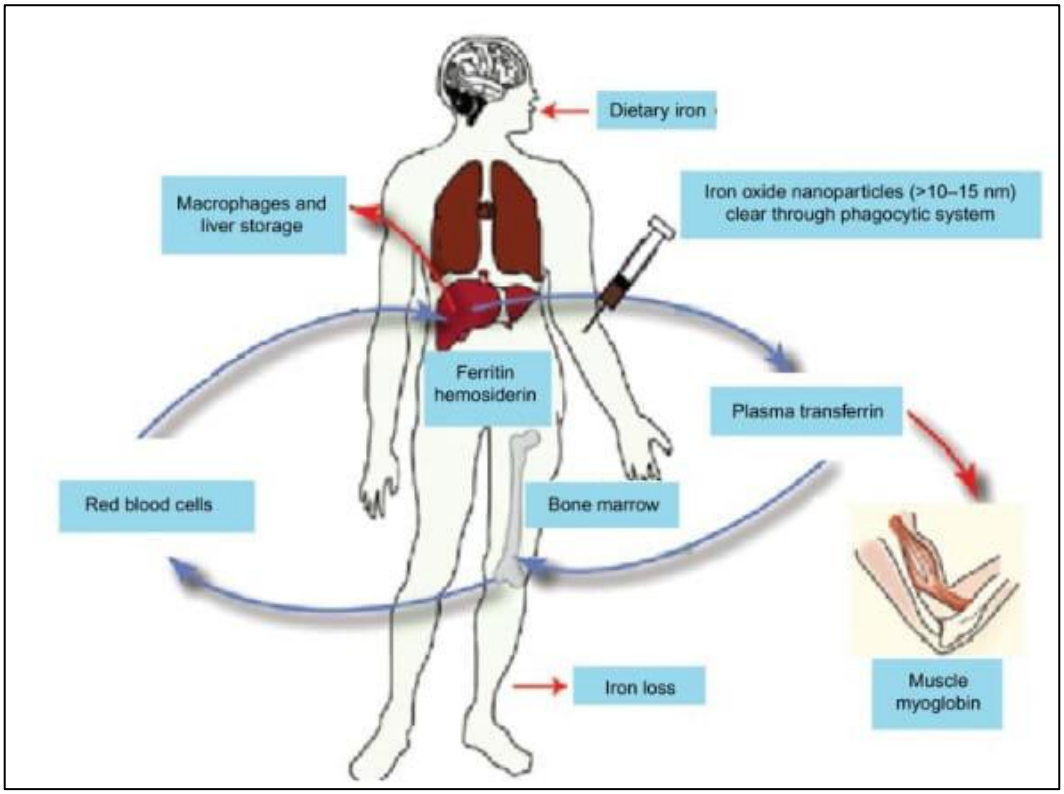


Figure 2 Biodistribution, Bioaccumulation and clearance of Iron oxide nanoparticles in human body

A very few studies report uptake and biodistribution of IONPs. Based on a study, the human body naturally contains magnetite and maghemite

The liver and spleen are the predominant sites for iron oxide bioaccumulation. In order to preserve equilibrium, the body regulates iron absorption, transit, storage, consumption, and recycling. This process is known as iron homeostasis. Disruptions in this balance can lead to iron deficiency Anaemia or iron overload conditions such as Hemochromatosis. Iron from food exists in two main forms: heme iron, is found in animal-based foods like meat, poultry, and fish, and it's the kind our bodies absorb most easily; and non-heme iron, found in plant-based foods such as beans, lentils, spinach, and whole grains. Dietary iron is absorbed in duodenum and proximal jejunum of the small intestine, mainly regulated by hepcidin harmone, produced by liver. Iron is oxidized to its ferric form (Fe^{3+}) after absorption and transport in bloodstream with the help of plasma protein transferrin. Transferrin protects free iron from accumulating within the circulation because unbound iron can cause free radicals to develop, which can harm organs and tissues. The levels of Hepcidin rise when the body contains sufficient iron, which inhibits ferroportin—the only known iron exporter in cells—from degrading it. Hepcidin production falls during periods of low iron levels or increased demand, which increases iron absorption and releases into the bloodstream. Our body stores most of its iron in the liver, spleen, and bone marrow, where it’s safely packed away in storage forms known as ferritin and hemosiderin. These forms act like reserve tanks, releasing iron when the body needs it for vital functions like making red blood cells. When our body needs it, iron travels through the bloodstream attached to a special transport protein called transferrin. Recently, the introduction of engineered iron oxide nanoparticles (IONPs) in biomedical applications such as contrast agent in magnetic resonance imaging (MRI), targeted drug delivery, treating iron-deficiency anaemia, and cancer therapy, has added a new dimension to our understanding of iron metabolism and its potential bioaccumulation pathways. IONPs typically of 10–15 nm in size, are introduced into the body via intravenous injection, oral administration, inhalation, intra-tumoral injection and topical or transdermal. After being administered, typically through an IV, they quickly make their way into the bloodstream and start spreading throughout the body. Their size, coating, and surface charge also influence the duration nanoparticles circulate within the bloodstream. When uncoated iron oxide nanoparticles (IONPs) enter the body, they are quickly recognized and taken up by the immune system—specifically by a group of cells known as the mononuclear phagocyte system (MPS), also called the reticuloendothelial system (RES). This system includes macrophages, which are immune cells that act like the body's cleanup crew. These macrophages are mainly located in the spleen, bone marrow, and liver. In the liver, a special type of macrophage called Kupffer cells plays a major role in

detecting and removing these nanoparticles from circulation, helping to keep the body safe from what it sees as foreign substances. Plasma protein (Ig complement protein) binds to the nanoparticle surface, forming protein corona which labels the nanoparticle as foreign. The macrophages recognize these opsonised particles and engulf them, breaking them down and releasing iron ions that enter the body's natural iron metabolism pathways. This phagocytic clearance mechanism is crucial for preventing the accumulation of nanoparticles in non-target tissues and potential toxicity. MPS involves major organs which includes the liver, spleen, lymph nodes, and bone marrow. Kupffer cells, that form the hepatic senusoids and serve as the main site for the clearance of nanoparticles, are found in the liver. The spleen acts like a natural filter for our blood, and one of its key jobs is done by special cells called splenic macrophages found in the red pulp. These cells help keep us healthy by removing old or damaged red blood cells and trapping tiny foreign particles, including nanoparticles. In this way, the spleen supports the immune system by cleaning the blood and protecting the body from potentially harmful substances. Smaller IONPs (<100 nm) with hydrophilic coating may evade immediate MPS uptake, whereas larger or positively charged particles are rapidly taken up.

Magnetic Resonance Imaging (MRI) is the most commonly used imaging modality and a non-invasive tool for cancer diagnosis. Contrast agents (CAs) create tissue contrast by magnifying the longitudinal (T1) and transverse (T2) relaxation rates. The use of iron oxide nanoparticles (IONP) as contrast agents for MRI is a better alternative than using gadolinium-based contrast agents due to their toxicity. Iron oxide nanoparticles are less toxic and more cyto-compatible and posses' magnetic properties.

In conclusion, iron metabolism in the human body is a complex and dynamic system that balances iron absorption, transport, storage, utilization, and loss. Plasma transferrin plays a central role in distributing iron to critical sites such as the bone marrow and muscles, while macrophages and the liver manage recycling and storage. Iron oxide nanoparticles represent a novel form of iron supplementation and therapeutic intervention, integrating into the natural metabolic cycle through phagocytic clearance and subsequent incorporation into cellular processes. However, careful consideration of their long-term effects and bioaccumulation patterns is necessary to harness their benefits safely. Understanding this intricate balance is crucial for managing iron-related disorders and for the continued development of nanoparticle-based biomedical applications.

1.6. Antibacterial activity of IONPs

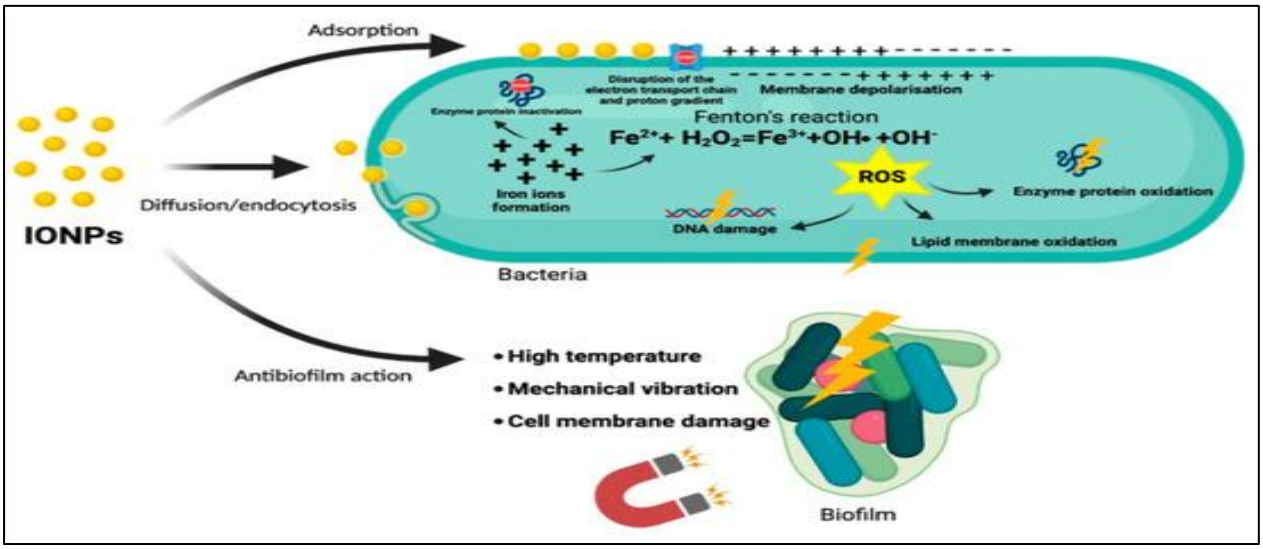


Figure 3 ANTIBACTERIAL ACTIVITY OF IONPs

Metal oxide interacts with bacterial cell membrane by electrostatic interaction which damages their membrane and induce toxic oxidative stress by the generation of Radial Oxygen Species (ROS). Metal ions are able to bind to mecapto (-SH), amino (-NH), and carboxyl (-COOH) groups of Proteins, including enzymes, which leads to inactivation or partial inhibition. IONPs have been found to effectively kill or inhibit bacterial growth which makes them potential antibacterial agent in biomedical and environmental applications. Their adsorption on bacterial cell surface plays a major role in their antibacterial mechanism, biosensing or biofilm disruption. The first step in the antibacterial action of IONPs is adsorption onto the bacterial surface which involves physiochemical interaction between the nanoparticles and bacterial cell envelope (either gram positive or gram negative).

Gram-positive bacteria (e.g., Staphylococcus aureus) has negatively charged surface due to Teichoic acid and Gram-negative bacteria contains lipopolysaccharides (LPS) which also gives negatively charged surface. Nanoparticles readily attach to the surface of bacteria due to the electrostatic interaction between the negative-charged bacterial cell membranes and the positively charged IONPs [50]. This adsorption interferes with the bacterial membrane's integrity and can lead to membrane depolarization, a condition where the normal electrical potential across the membrane is disrupted, which increases cell permeability causing leakage of cytoplasmic content and essential ions collapsing the membrane potential and ultimately cell lysis. This depolarization impairs the function of transmembrane proteins and disrupts the proton motive force, which is essential for ATP

production and nutrient transport in bacteria. Reactive Oxygen Species (ROS) generation through Fenton like reaction, induces oxidative stress which damages cellular components such as DNA, proteins and lipids which leads to cell death.

ROS are known to inflict widespread cellular damage. Within bacterial cells, ROS leads to the oxidation of lipids, proteins, and DNA. Lipid peroxidation is a chain reaction of free radicals attacking unsaturated fatty acids that compromises the structural integrity of cellular membranes, making them more permeable and prone to rupture. This reaction can be terminated by the reaction of peroxyl radical with each other and antioxidants. Protein oxidation alters the three-dimensional structure of essential enzymes and structural proteins, rendering them non-functional. DNA damage, including strand breaks and base modifications, impairs replication and transcription, leading to genetic instability and eventual cell death. Together, these effects disrupt core cellular processes, rendering the bacteria unable to survive or proliferate.

2. AIMS AND OBJECTIVE

- Green Synthesis of nanoparticles from *Pongamia pinnata* and $FeCl_3$
- Characterisation of the synthesised nanoparticle
- Application of the green synthesised Iron Oxide Nanoparticles

3. LITERATURE REVIEW

Nanotechnology is the Science and Engineering of the materials at the atomic and molecular level, that comes in range of 1-100 nm, having large surface area to volume ratio, that increases their overall reactivity and functionality. Classification of nanomaterials are based on the substrate type such as carbon-based, metal based, ceramic nanoparticle, lipid based, and polymer nanoparticles are some most studied types of nanoparticles. Nanoparticles have various application across diverse fields such as mechanics, optics, electronics, biotechnology, microbiology, environmental remediation, medicine, engineering and materials science.

Numerous physical and chemical methods have been employed for the nanoparticle synthesis of metal and metal oxide nanoparticle that requires use of toxic chemicals which makes it detrimental for environmental, plants and animal application. Physical methods like thermal decomposition, laser ablation, inert gas condensation (IGC), physical evaporation, electric arc discharge, and sputtering are used to transform bulk materials into nanoparticles. There are several examples of chemical methods employed for the nanoparticles synthesis such as hydrothermal processing, chemical vapor deposition, sol gel techniques, and co precipitation method. [18] The different factors such as pH, temperatures, and humidity affect the physiochemical characteristic and size of the nanoparticle.

Table 2: Comparison of different physiochemical techniques

Sl. No.	TECHNIQUES	ADVANTAGE	DISADVANTAGE
1.	Physical Vapor Deposition (PVD)	● High Purity ● Precise Control	● Requires vaccum system ● Not for large scale
2.	Ball Milling	● Low temperature processing ● Large scale production ● Doesn’t require high temperature or solvent	● Long processing time ● Agglomerates formation ● Residual strain in the nanoparticle due to mechanical strain
3.	Laser Ablation	● Cost effective for large scale production ● Generates Minimal waste ● Versatile material compatibility	● Limited production rate for large scale production ● Agglomeration and aggregation control is difficult
4.	Sputtering	● Maintains the	● High Equipment

		stoichiometry of the target material ● Uniformity	Costs, Target Material cost ● Despite operating in a high vacuum, sputtered films can be susceptible to contamination
5.	Electron beam lithography	● Well controlled interparticle spacing	● Requires expension and highly complex machine
6.	Sol Gel method	● Produces nanoparticles at relatively low temperatures (25-200 °C) ● Produce highly homogenous material	● Difficult to obtain large monolithic pieces due to shrinkage during drying ● Challenging to eliminate residual porosity and hydroxyl groups
7.	Co precipitation method	● Precise control over the size and composition ● Produce nanoparticles with good crystallinity	● Time consuming ● Agglomeration ● Microscopic inhomogeneity
8.	Hydrothermal	● Uniform size distribution and high crystallinity. ● Relatively inexpensive ● Easy surface modification with functional ligand during synthesis	● It requires specialised equipments such as autoclaves or sealed reactors ● Difficult to directly observe the crystal growth process
9.	Electric arc discharge	● High yield ● High temperature and Joule heating effect in the arc discharge process can lead to self-crystallization	● Difficult in controlling stable arc ● Inhalation of carbon nanotubes produced by arc discharge have health implications
10.	Inert Gas Condensation (IGC)	● High purity and uniform characteristics ● Scalable method, demonstrated by surface engineered IONPs. [19]	● Requires high vaccum facilities ● Limitation in producing porous material

Although aforementioned methods are promising and large-scale production methods, but they involve use of toxic chemicals and require great infrastructure for nanoparticles production along

with discharge of toxic byproducts as well as usually unsafe for biomedical applications. Researchers are emerged in the finding of a cost effective and safe for biomedical use methods.
Green synthesis utilises eco-friendly and cost-effective biological sources as reducing and stabilising agent without the intervention of toxic chemicals making the nanoparticle most suitable for biomedical application. Various biological sources such as bacteria, fungi, viruses, actinomycetes, biochemical agents (such as ascorbic acid, amino acid, haemoglobin, and myoglobin, synthetic tannin and gallic acid) have been employed for the synthesis of IONPs.

Table 3: List of the plant parts and precursor used in literature for IONPs production

SL. NO.	BIOLOGICAL SOURCES	PART USED	IRON PRECURSOR
1.	*Azadirachta indica* (Neem)	Leaf	$FeCl_2.6H_2O$
2.	*Cinnamomum camphora*	Bark	$Fe(NO_3)_3.9H_2O$
3.	*Lagenaria siceraria*	Leaves	$FeCl_3.6H_2O$
4.	*Moringa oleifera*	Leaves	$FeSO_4$
5.	*Camellia sinensis*	Leaves	$FeCl_3$
6.	*Citrus sinensis*	Peel	$Fe(NO_3)_3$
7.	*Ocimum sanctum* (Tulsi)	Leaves	$FeCl_2.4H_2O$
8.	*Syzygium cumini*	Seeds	$FeCl_3$
9.	*Zingiber officinale* (Ginger)	Rhizome	$FeSO_4$
10.	*Trigonella foenum-graecum* (Fenugreek)	Seeds	$FeCl_3$
11.	*Allium sativum* (Garlic)	Bulb	$FeCl_3$
12.	*Punica granatum* (Pomegranate)	Peel	$FeSO_4$

The nanomaterial synthesised from micro-organism is less mono-dispersed and the synthesis rate is slow when compared to plant based synthesis. Plants certainly produces more stable nanoparticle and best biological source for fast and large scale synthesis as compared to microbial source.
Kuang et al synthesised nanoparticle using three different types of tea leaf extract (green tea, oolong tea, and black tea) separately and used the nanoparticle as catalyst for Fenton like oxidation of Monochlorobenzene (MCB) observing three different degradation rate of 69% for GT-Fe-Np, 53% for OT-Fe-Np and 39% by BT-Fe-Np. As compared to conventional Fenton reaction, Fenton like reaction from phyto-synthesised nanoparticle takes place in more sustainable manner. The plant mediated Fe-Np acts as Fenton Catalyst with H_2O_2.

Eucalyptus mediated Iron Polyphenol (Fe-P-Np) complex were potential in degradation of dye and adsorption flocculation against Acid Black 194 dye at 25°C. Around 100% dye was decolourised and 87% removal of total organic carbon was achieved.
Huang et al reported the decline in FeNP concentration with an increase in plant leaf extract due to decrease of Fe^{+2} concentration and studied their reactivity for MG dye degradation. The reactivity was observed to be influenced by pH and temperature. Luo et al studied FeNP synthesised by grapes leaf extract for degradation of Acid Orange (II) dye and the nanoparticle complex was proved to be more effective than the extract alone. Priyabrata et al reported synthesis of iron oxide nanoparticles using pigments extracted from neem leaves through Soxhlet extraction. They went on to test how well these nanoparticles could break down and adsorb methylene blue dye in a sodium hydroxide solution, demonstrating their potential for environmental cleanup applications

Rao et al evaluated Fe^0/Fe_3O_4 nanoparticle and yeast cells nanocomposite has been shown to have capacity to remove hexavalent Chromium and proved to be a good biosorbents. Madhavi et al reported synthesised zero valent iron nanoparticle using Eucalyptus globules leaf extract and used it adsorption of Cr (VI) metal. Savasari et al synthesised ZVINp by Ascorbic acid and used it for Cd (II) reduction from aqueous and ascorbic acid.

Zheng et al studied nanoparticle induced ferroptosis which is an iron dependent cell death mechanism and discussed the nanosized inducer and the cascade signal in cells. Vladimir et al reported the use of IONPs to reprogram macrophage responses and demonstrated that it can inhibit the migration and invasion of tumour cells when coated with polyethyleneimine (PEI). Nanoparticle can reduce its effectivity and literature shows plant synthesised FeNp has been successfully stabilised by solid supports such as polymers, zeolites, silica, etc., and used potentially in the degradation of pollutants and pollution remediation.
R. Anuradha et al. conducted a GC-MS analysis of the ethyl acetate extract from *Pongamia pinnata* flowers and identified 30 bioactive compounds based on peak area, molecular weight, and molecular formula. Notable compounds detected include dibutyl phthalate ($C_{16}H_{22}O_4$), oleic acid ($C_{18}H_{34}O_2$), nonadecene ($C_{19}H_{38}$), 1-docosene ($C_{22}H_{44}$), oxalic acid, and allyl hexadecyl ester ($C_{21}H_{38}O_4$), with corresponding retention times (RT) of 18.49, 21.92, 14.93, and 10.77 minutes, respectively. With peak areas of 0.25 and 0.19, respectively, 1-hexyl-3-methly-(C12H24), 1-cyclopentane, and 1-cyclohexylnonene exhibited the lowest component concentrations in the flower extracts.
Bannoth at al (2014) used *Pongamia pinnata* Leaf Extract Biosynthesis of Silver Nanoparticles to Serve as a Successful Larvicide on Dengue Vector *Aedes albopictus* (Skuse) (Diptera: Culicidae).

Sundrarajan et al (2015) reported synthesis of zinc oxide nanoparticle using Pongamia leaf extract and studied the antibacterial effect against pathogenic bacteria such as *E. coli* and *S. aureus*. Raut et al (2010) also reported the extracellular synthesis of silver nanoparticle using *P. pinnata* dried leaf and investigated their anti-bacterial effect against *S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae.*

C M Noorjahan et al (2024) demonstrated the use of P. pinnata leaf in the biosynthesis of CuO nanoparticle by grounding the air dried leaf and boiling it in distilled water to prepare the plant extract. They also performed anti-bacterial test on both gram positive and gram negative bacteria using agar well and disc diffusion assays.

Several studies confirmed the pharmacological activities of *P. pinnata* such as anti-microbial, anti-parasite, anti-inflammatory, anti-diabetic, anti-helminthic, cardio-protective activity etc. *Pongamia pinnata* has been traditionally used as curative agent for various diseases. Only one study has been reported on pharmacokinetic study of this plant.

The presence of various bioactive chemical constituents has been confirmed from the literature. Flavonoids have been the most common of all.

4. MATERIALS AND METHODS

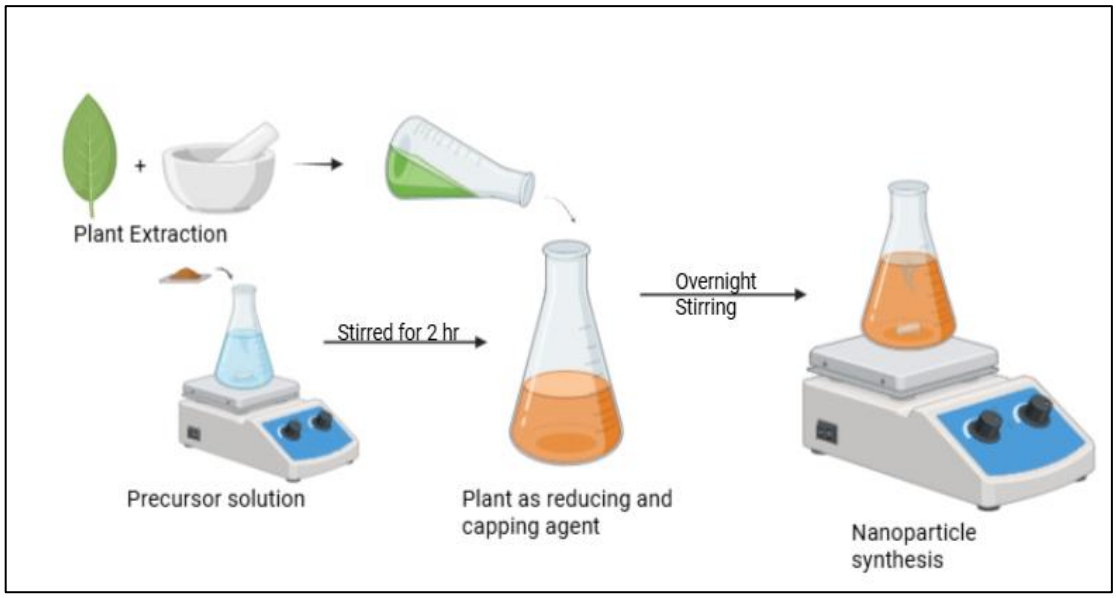
4.1. Materials:

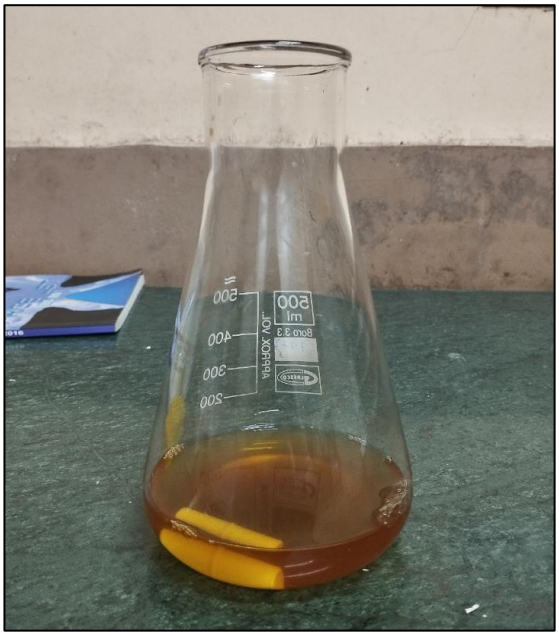
Iron (III) Chloride ($FeCl_3$) was procured from Sigma-Aldrich (Product No. 157740, reagent grade, 97%), *Pongamia pinnata* leaf was collected from IIEST, Shibpur campus, MTT reagent (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) was obtained from Thermo Fisher Scientific (Catalog No. M6494) and used as per the manufacturer's instructions, PBS (Phosphate Buffered Saline), DMSO (Dimethyl Sulfoxide) solution, Luria-Bertani (LB) agar (Lennox formulation) was obtained from Sigma-Aldrich (Product No. L5542), comprising 10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, and 15 g/L agar

4.2. Plant Collection and Extract Preparation:

In this method, plant leaves serve a dual purpose—they help convert metal ions into nanoparticles (acting as a natural reducing agent) and also coat the particles (acting as a capping agent), which enhances their stability and makes them more compatible with biological systems. The medicinal plant *Pongamia pinnata* leaf was collected from IIEST (Shibpur) green campus. Further, the leaves were precisely rinsed and purified using deionized water to remove any surface contaminants before being allowed to air dry in shade. After complete drying in room temperature, the plant material was grinded into a fine powder using mortar and pestle. Subsequently, the plant extract was biosynthesized by adding 10 g of the material with 20mL of distilled water, and the bioactive compounds were extracted using ultrasonic assistance and freeze fracture. To preserve its valuable content heat is avoided. The extract was passed through blotting paper to remove solid residues, then kept at 4°C to preserve it for later use.

4.3. Green Synthesis of IONPs:





The green synthesis of IONPs using leaf extract involves mixing the plant extract with a solution of iron precursor such as $FeCl_3$. We used 10mM of Anhydrous $FeCl_3$ [Mol wt. 162.2] solution was prepared (162.2 mg in 100 mL D.W) and stirred with the help of a magnetic stirrer under absence of light condition.10mL of filtered Plant Extract was added. The final solution was allowed to continuously stirred with the help of a Magnetic stirrer for 24 hr. The reaction in which iron ions are reduced by plants bioactive compound in the extract. The hydroxyl (-OH) groups present in flavonoids structure, donate electrons to iron ions forming iron atoms which forms iron oxide nuclei. The functional group such as Hydroxyl, Carboxyl, and Amine groups in bioactive compounds interact with IONPs surface forming protective layer and improve its biocompatibility.

4.4. Characterization of the prepared IONPs:

Various techniques were employed to examine the size, structure of the synthesised nanoparticles.

4.4.1. UV-Visible Spectroscopy

Based on the Beer-Lambert rule, UV-vis spectroscopy is an established method for analyzing the optical and structural characteristics of nanomaterials. By using a UV-visible spectrophotometer with absorbance measured at wavelengths.

4.4.2. Dynamic Light Scattering

Dynamic Light Scattering (DLS)—also referred to as quasi-elastic light scattering or photon correlation spectroscopy—is widely used to determine the size of nanoparticles in liquid, specifically their hydrodynamic diameter and surface charge (zeta potential). In this technique, a monochromatic laser is aimed at a nanoparticle suspension, causing the light to scatter in different directions as it interacts with the particles in the solution. As the light interacts with these particles, it scatters in multiple directions. The way the light scatters varies depending on the particles' size and shape, which allows researchers to assess their physical properties with precision. The hydrodynamic size represents the size of the nanoparticles along with its surrounding hydration layer and zeta potential indicates the surface charge which is the measure of Nanoparticle stability in solution. DLS is not only useful for measuring nanoparticle size, but it also helps track how stable the particles remain over time. This is especially important when predicting how nanoparticles might perform in various environments or practical uses. To assess their surface charge—known as zeta potential—researchers often rely on techniques like electrophoretic and electroacoustic measurements. [15]

4.4.3. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was carried out at the Device Characterization Laboratory (Char Lab-I) in the School of Advanced Materials, Green Energy, and Sensor Systems at IIEST, Shibpur. This method helps identify the different functional groups on the surface of iron oxide nanoparticles (IONPs), revealing the presence of organic molecules attached to them. The technique measures how much infrared light the sample absorbs. On the graph produced, the X-axis shows the infrared wavelengths, while the Y-axis indicates how much light is absorbed or transmitted. The peaks, or absorbance bands, represent the vibrations of atoms in the sample as they respond to the infrared radiation, providing detailed information about its molecular structure. Higher wavenumber corresponds to higher energy vibrations, such as O-H, and C-H stretching.

4.4.4. X-Ray Diffraction

X-ray diffraction (XRD) is a non-destructive technique used to analyse the crystallographic structure of a material without damaging them. The typical wavelength of X-rays is 1 Å. X-ray beams are directed through the sample because their wavelength is similar to the spacing between the atoms. X-ray hits the atom and gets scattered in all directions. The angle of diffraction is measured to determine the difference between atomic planes using Braggs law,

$$\sin \theta = n\lambda /2d$$

where, θ is the angle of diffraction; λ is the wavelength added and d is the distance between atomic planes.

To analyse the structure of synthesised IONPs, XRD pattern was recorded on X-Ray diffractometer at range of 2θ from 10º-80º with a scan rate of 20º/min using Cu Kα (λ = 1.5406 Å) radiation with an accelerating Voltage of 40 KV.

4.5. Antimicrobial Test

3g of pre-mixed agar powder is dissolved in 100 mL distilled water and heated to dissolve completely and autoclave at 121°C for 15 min. The prepared broth is allowed to cool and poured to the plates and let them solidify. ***Escherichia coli*** optimally grows at 37°C under aerobic conditions and survives at temperatures of 4°C for extended periods of time on solid media. The glass spreader is sterilised by soaking in 70% ethanol for 1 minute, pass the spreader through flame to burn off excess ethanol. Allow the glass spreader to cool, swab the agar plate with the ***E. coli*** and allowed to grow for 24 hr. To access antibacterial activity of the synthesised IONPs, disc diffusion method, also known as Kirby-Bauer method was employed. The sterile Whatman No.1 paper disc of 5 mm dimension, containing known concentrations of 20 µg/mL to 150 µg/mL IONPs were placed on the inoculated agar plate along with a control disc. After incubation for 24 hr at 37°C, zone of inhibition was examined around the discs, where bacterial growth is inhibited. ImageJ software was used to measure the diameter of zone of inhibition. The average of 8 different diameters from a zone of inhibition was taken for different concentration of IONPs.

4.6. MTT Analysis

Cell viability analysis was performed on human cervical cancer HeLa cell lines using [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] MTT Assay. Cell seeding was done in 96 well plate and incubated overnight at 37°C, 5% CO_2 to allow cells to attach. Cells were treated with different concentrations of IONPs, plant extract, green IONPs and a control of untreated cells were kept for 24-72 hours of incubation. 5 mg/mL MTT solution was prepared in PBS and 10 µL was added to each well and incubated for 3 hours at 37°C. Yellow tetrazolium crystal gets converted into purple formazan crystal in viable cells due to reduction reaction after incubation. Absorbance is measured at 570 nm using microplate reader and cell viability % is calculated using following formula:

$$\text{Cell Viability (\%)} = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ control\ cells} \times 100$$

5. RESULTS and DISCUSSION:

5.1. UV Visible Spectroscopy Analysis

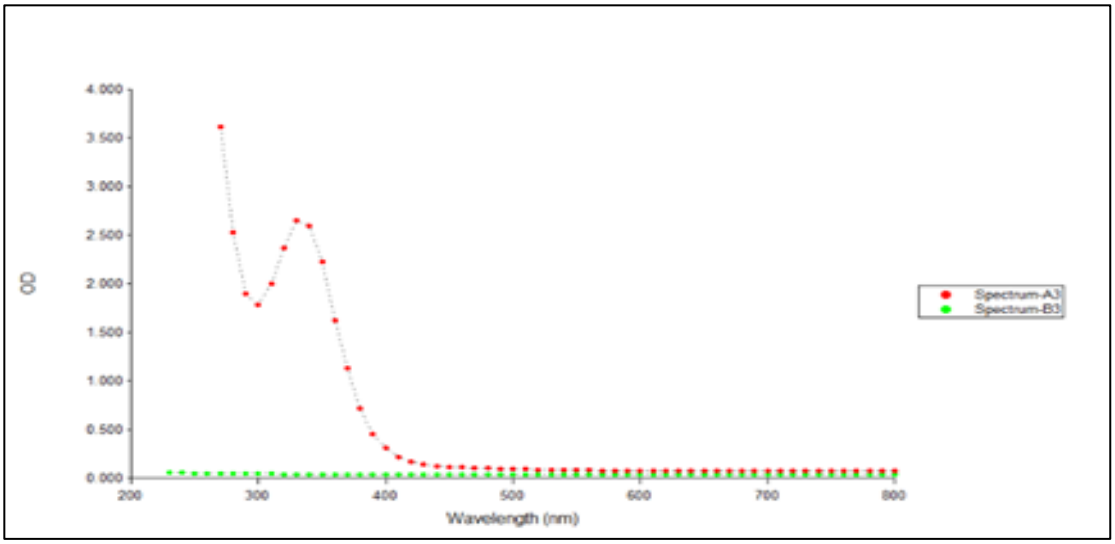


Figure 4 Characterisation of IONPs using UV Visible spectroscopy

The formation of the green synthesized IONPs was confirmed using XYZ UV-Visible Spectroscopy and characteristic surface plasmon resonance (SPR) peak was observed at 370 nm which is typical for iron oxide nanoparticle. No peak beyond 400 nm suggests minimal aggregation and good dispersion of the nanoparticles.

5.2. Dynamic Light Scattering (DLS) Analysis

DLS analysis was conducted to determine the hydrodynamic diameter, size distribution, surface charge (Zeta Potential) and polydispersity index (PDI).

5.2.1. Z-average (d-nm):

Zeta average is the cumulants mean diameter and represents hydrodynamic diameter of the nanoparticle. It is intensity weighted which means that the larger particles influence it more even if they are few in number. It represents the average size of particle as it moves in the solution. The average size of the nanoparticle is 98.52 nm.

5.2.2. Zeta Potential:

The zeta potential value measures the surface charge of the nanoparticle synthesized was recorded as -39.4 mV. A zeta potential above ±30 mV typically indicates good stability due to electrostatic repulsion between particles. A higher absolute zeta

potential indicates better stability and aggregation is prevented. The obtained value suggests the nanoparticle has negative charge on surface, likely coated with negatively charged groups (eg- -OH, -COOH), is relatively stable in the suspension and shows a very good colloidal stability with low aggregation risk which means that the suspension will remain dispersed for long time.

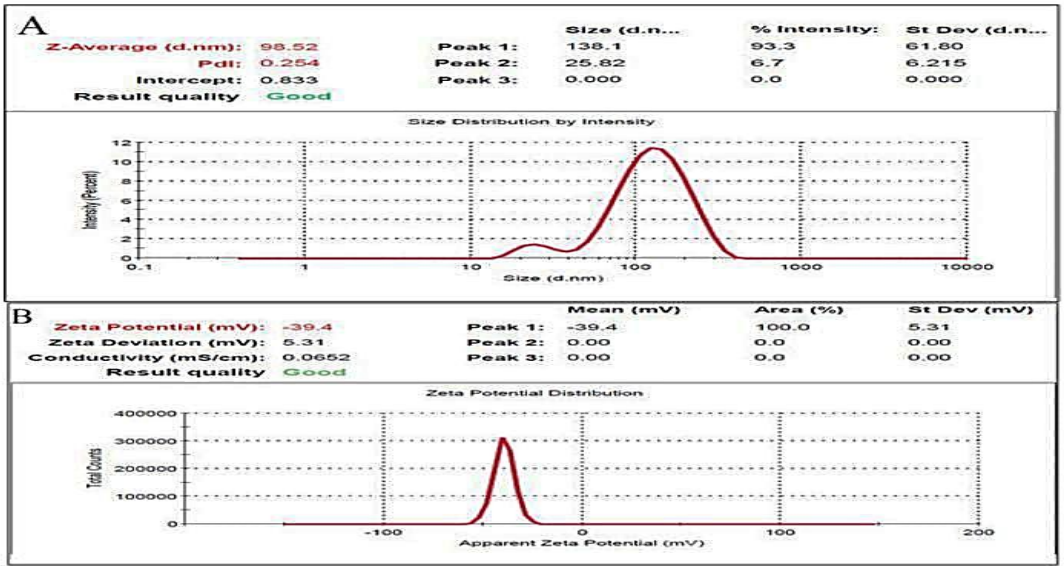


Figure 5 DLS analysis showing the (A) Hydrodynamic size distribution and average particle size

5.2.3. Polydispersity Index (PDI):

The polydispersity index (PDI) is used to describe how uniform the particle sizes are within a sample. A lower PDI indicates that the particles are mostly similar in size, while a higher value suggests a broader size distribution and greater variability. This value close to 0 indicates a highly monodisperse particle; value around 0.1 is generally considered acceptable; but a value between 0.2 and 0.5 is an indication of moderate polydispersity. The PDI value was found to be 0.254, indicating the uniformity of particle size distribution. A PDI value below 0.3 is generally suggests that the nanoparticles in the solution are relatively uniform in size, a monodisperse system. The observed value suggests that the synthesized nanoparticles have a uniform and stable size distribution. With this level of uniformity, the particles are less likely to clump together or behave inconsistently.

5.3. Fourier Transform Infrared (FTIR) Spectroscopy:

The FTIR Spectroscopy analysis to identify the functional group present on the IONPs synthesized from *P. pinnata* extract. The FTIR spectrum displays transmittance (%) on the Y-axis and wavenumber (cm^{-1}) on the X-axis. The FTIR spectra showed characteristic peaks in the range of 4000-650 cm^{-1}. A simple spectrum with fewer than five peaks generally suggests the presence of small organic molecules or straightforward inorganic compounds. The single bond region of 4000-2500 cm^{-1} includes stretching vibrations of O-H, N-H, and C-H bonds.

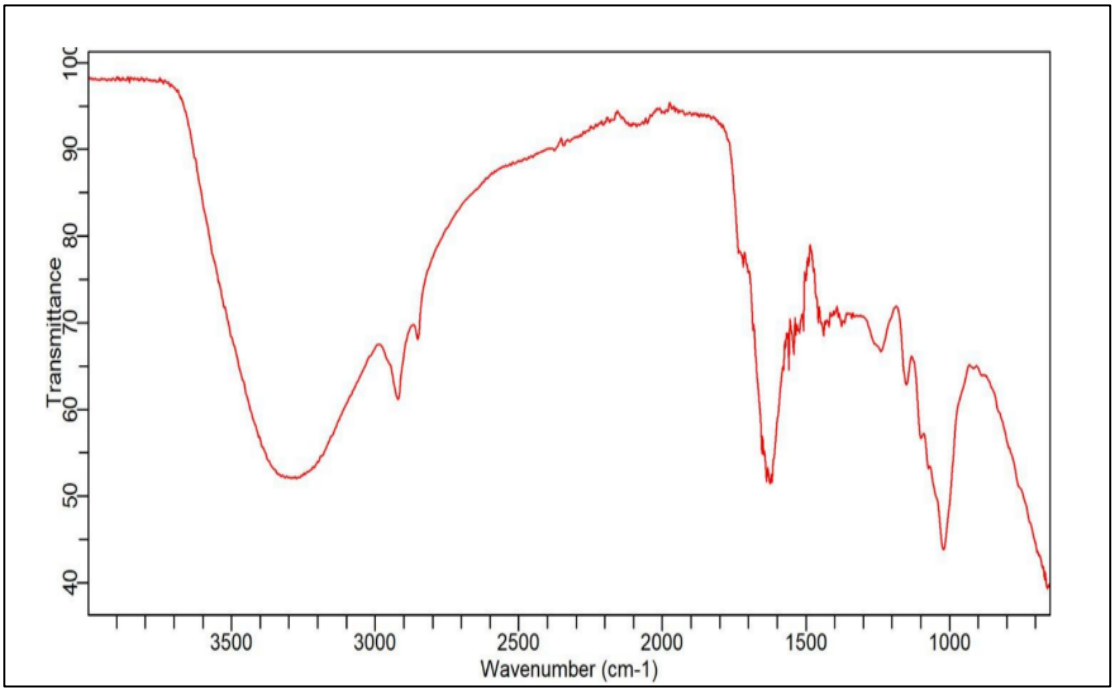


Figure 6 FTIR spectrum of IONPs synthesised using Pongamia pinnata leaf extract confirming the presence of functional group involved in nanoparticle synthesis and stabilisation

A broad and strong absorption band around 3400 cm^{-1} corresponding to O-H and N-H stretching vibrations indicating presence of phenolic compound and alcohol from polyphenols and proteins which may act as reducing agent. The absorption peaks at ~2900 cm^{-1} were attributed to the C-H stretching. The triple bond region around 2500-2000 cm^{-1} corresponds to C ≡ C (alkyne) and C ≡ N (nitrile) stretching. The C ≡ C peak is weak due to low polarity whereas C ≡ N shows stronger absorption. The infrared region between 2000 and 1500 cm^{-1} is primarily characterized by stretching vibrations from carbonyl groups (C=O) and carbon-carbon double bonds (C=C) found in alkenes and aromatic rings. For instance, carbonyl groups in compounds like ketones, aldehydes, esters, and acids typically

show strong absorption peaks near 1680 cm^{-1}.

Table 4: Result interpretation of FTIR Spectra Graph

Wavenumber Range (cm^{-1})	**Probable Functional Group**	**Compound Class**
3200-3400	O-H Stretching	Phenols, Alcohol, Polyphenols
~3300	N-H Stretching	Amines
~2900	C-H Stretching ($-CH_2$)	Alkanes or Saturated Lipid
~2800	C-H Stretching ($-CH_3$)	Alkanes or Fatty acid
~1600	C=C Stretching	Alkenes, Aromatic rings
~1500	C=O (Carbonyl) stretching	Amide I
~1300	C-N Stretching	Amide
~1100-1300	C-O Stretching	Alcohols, Ethers
~1000	C-O-C Stretching	Ester and Ether Linkage

The region between 1500 and 500 cm^{-1} in an FTIR (Fourier Transform Infrared) spectrum is called the "**fingerprint region**" because the absorption pattern in this range is unique to each molecule, much like a human fingerprint is unique to each person. This part of the spectrum is more complex and contains unique absorption patterns that reflect the overall molecular structure, making it especially useful for identifying specific compounds. A strong peak around 1000 cm^{-1} typically represents C-O stretching vibration. The appearance of small broad bands at around 850-900 cm^{-1}, out of plane bending vibrations of aromatic C-H bonds, which are characteristic of substituted benzene rings. These functional groups are likely derived from the plant extract and are responsible for the reduction and stabilization of nanoparticles, indicating successful green synthesis.

5.4. X-Ray Diffraction (XRD) Analysis

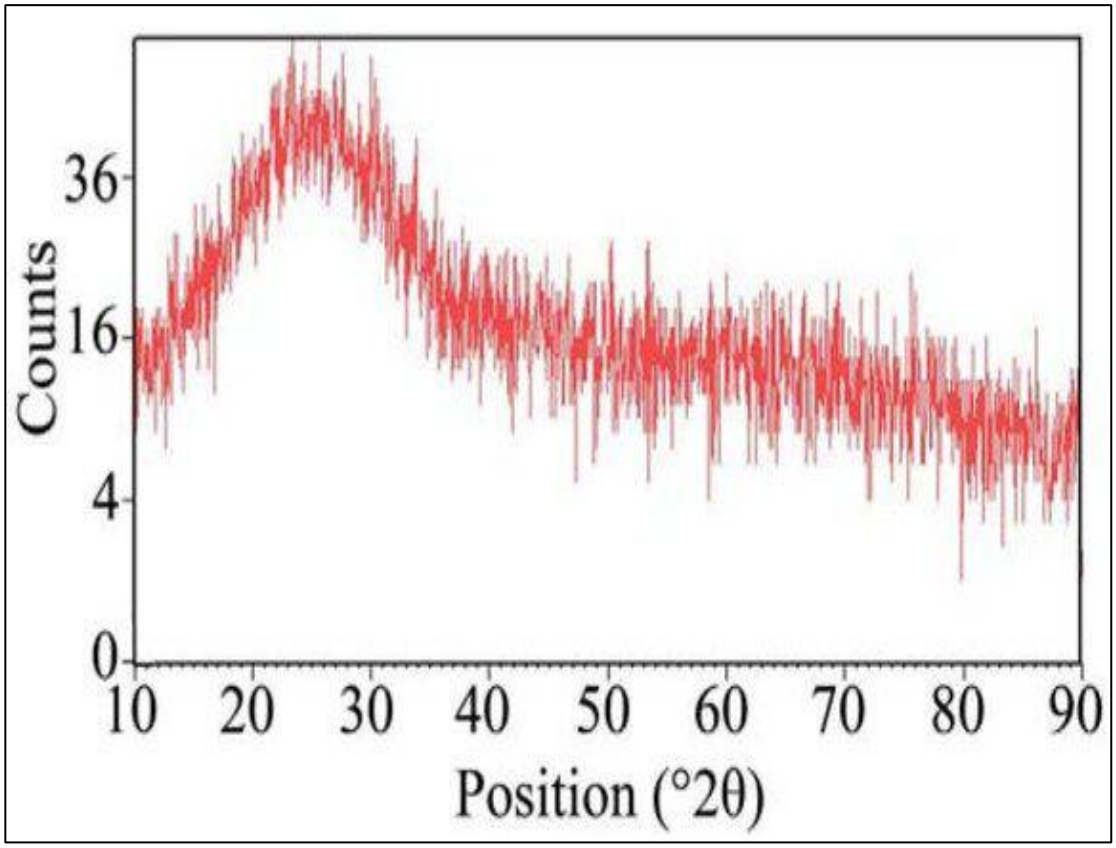


Figure 7 XRD Analysis

X-Ray Diffractometer (MAKE: BRUKER; Model: D8 Advance) from Department of Metallurgy and Materials Engineering at IIEST, Shibpur was used for texture analysis.
The XRD pattern of the green synthesized IONPs exhibited no distinct peaks corresponding to the amorphous nature of the particles. The characteristic diffraction peaks were observed at 2θ values of 20º-40º, which are in agreement with the standard diffraction data of Iron Oxide Nanoparticles. Nanoparticles contain far fewer atoms compared to bulk materials, so the regular arrangement of atoms (lattice) is less extensive. As a result, instead of sharp diffraction lines, the X-ray diffraction patterns show broader peaks. In fact, the smaller the particle size, the more these diffraction peaks tend to widen. The 2θ values of 20º-40º region corresponds to particle with low angle scattering such as in amorphous oxides. The low intensity of 36 counts confirms the poor crystallinity due to absence of long-range periodicity. The broad hump results from overlapping of diffuse scattering from multiple disordered domains or nanoparticle interfaces.

5.5. Cell Viability Analysis

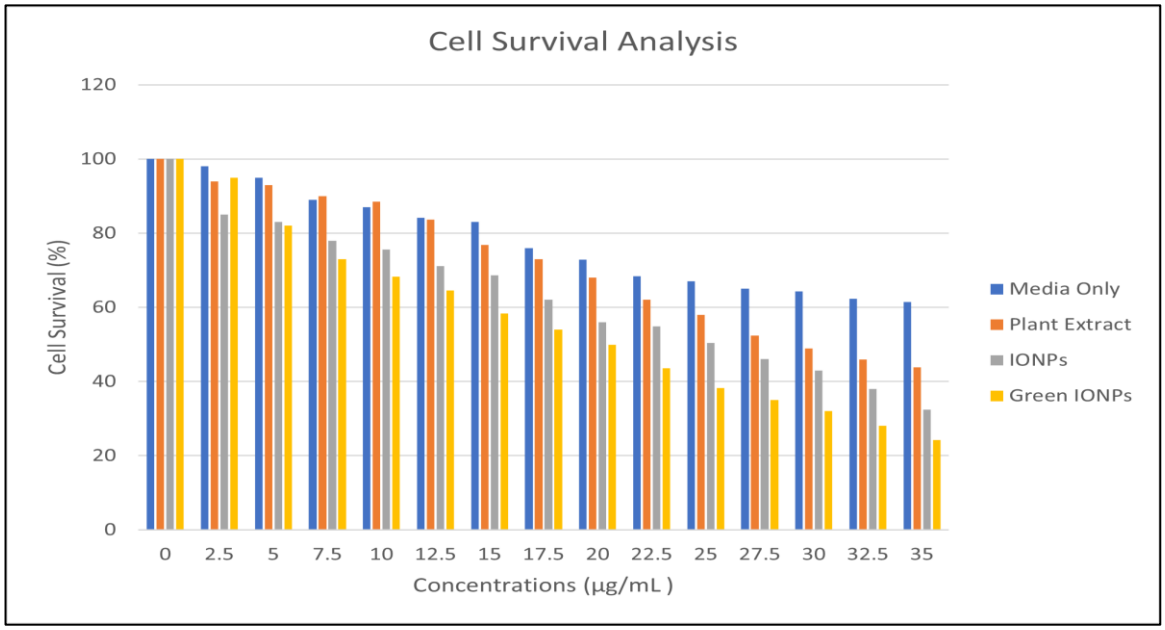


Figure 8 MTT Analysis was done on HeLa cells

Cell viability test was done on HeLa cell lines with media containing plant extract, IONP and green synthesised IONP using MTT assay. The dose response was constructed and the IC_{50} values were determined from the fitted sigmoidal curves. As shown in figure, the cell survival decreases in a concentration dependent manner upon IONPs treatment. The control containing only media shows ~100% cell viability across all concentrations, indicating the culture condition was nontoxic. Treatment with plant extract shows minimal toxicity after the control media, suggesting that the plant extract itself doesn't contain any toxicity. Chemically synthesised IONPs demonstrated moderate toxicity to the cell with a progressive decline in cell viability with increasing concentration. The green synthesised nanoparticle shows significantly higher cytotoxicity with the IC_{50} value of approximately 20.71 µg/mL compared to both plant extract (IC_{50} = 40.05 µg/mL) and chemically synthesised nanoparticles (IC_{50} =30.69 µg/mL).

5.6. Antimicrobial Test:

The antimicrobial potential of the green synthesised nanoparticles is evaluated by the disc diffusion method, showing zone of inhibition on the region of higher concentration. A zone of inhibition is an area where the bacterial growth is ceased due to the anti-bacterial activity of the compound and its inhibitory effect is studied. The zone of Inhibition was measured using a

free, open-source image processing software, ImageJ developed by National Institute of Health (NIH). Measurements of the zone of inhibition were taken at eight separate positions using the software, and the average diameter was then calculated. The average diameter of the zone of inhibition at 20 µg/mL concentration of IONPs was measured to be 11.35 mm, at 50 µg/mL it was 20.00 mm and maximum at 150 µg/mL it was 24.32 mm, this suggests a dose dependent antibacterial effect.

Table 5: The average diameter of the Zone of Inhibition at different concentration of IONPs

Concentration (µg/mL)	20 µg/mL	50 µg/mL	150 µg/mL
Zone of Inhibition	11.35 mm	20.00 mm	24.32 mm

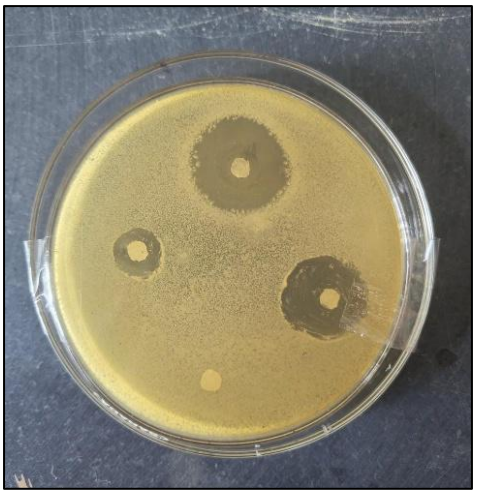


Figure 9(a) Antimicrobial Analysis

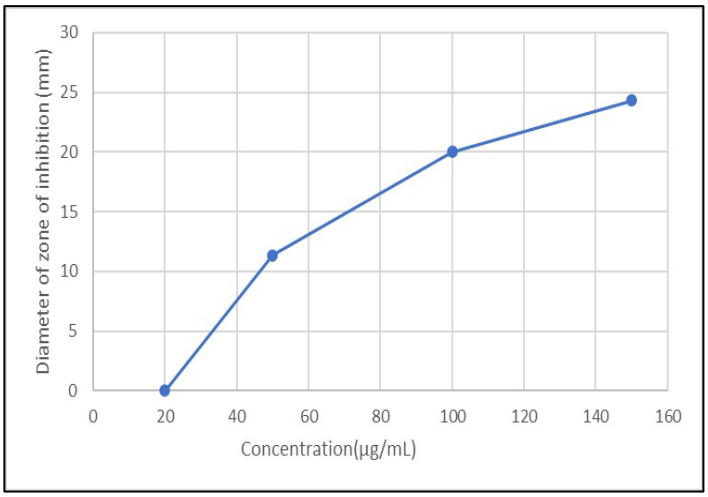


Figure 9(b) Dose-Response curve

6. CONCLUSION

In conclusion of the research undertaken, the green synthesis of iron oxide nanoparticles using plant leaf extract has been found to be an excellent eco-friendly and facile route. The leaf extract of *P. pinnata* were confirmed as a dual reducing and capping agent. The characterized property of the nanoparticle suggests their promising potential for various biomedical, environmental and drug delivery applications.

5.1. Green Synthesis of nanoparticles

The green approach in the synthesis of nanoparticles represents a paradigm shift in nanotechnology by integrating eco-friendly principles with advanced material science. In this study plant leaf extract is explored as a dual reducing and capping agents for nanoparticle synthesis eliminating the requirement of hazardous chemicals, high temperature and complex procedure as of conventional techniques. This method utilizes readily available natural materials which makes it eco-friendly, cost effective, biocompatible and safer approach. Despite the advantages, there are certain limitations which needs to be addressed such as lack of standardization due to variability in the biological extract. The exact mechanism and biochemical pathway are yet unclear. The menace of aggregation or degradation over time without proper storage condition is inevitable. The environmentally conscious approach of synthesizing nanoparticles through ‘green’ techniques is a novel shift in the world of nanotechnology as it cares for biological and ecological problems usually associated with the conventional physical and chemical methods of the nanoparticle fabrication. It follows the tenets of green chemistry by employing biological materials such as plant extracts, bacteria, fungi, and algae as reducing and stabilizing agents in the design of nanoparticles. In addition to eliminating the use of harmful chemicals, this method employs safe, efficient and eco-friendly processes. Their attention to sustainability has increased interest in, and concern for, ecological footprints, making green approaches to the synthesis of nanoparticles an attractive domain within material and environmental science, biotechnology and engineering.

5.2. Applications and Implications of IONPs

5.2.1. Magnetic Resonance Imaging (MRI) Contrast Enhancement Using Green-Synthesized Iron Oxide Nanoparticles (IONPs)

Increasing the efficacy of Magnetic Resonance Imaging (MRI) is one of the green-synthesized nanoparticles biomedically applied which MRI scans, done without any tissue injury, utilize to locate soft tissues like tumors. Iron oxide nanoparticles (IONPs) have gained wide acceptance as one of the best T2-weighted contrast nanomaterials, because of their superparamagnetic properties, which greatly lower the transverse relaxation time (T2) and heighten the negative contrast in MR images. Cytotoxicity of the green synthesized nanoparticles is reduced due to elimination of toxic chemicals during synthesis and natural capping of reactive functional groups (such as hydroxyl, carboxyl, and amino) can be supplied by biomolecules derived from green synthesis for additional surface modification or targeting. Natural surface coatings help the mononuclear phagocyte system (MPS) recognize and metabolize substances, facilitating a safer removal from the body.

A notable example of targeted imaging is the conjugation of IONPs with anti-HER2 antibodies, enabling targeted imaging of HER2-positive breast cancer cells. The specificity of antibody-antigen binding allows the nanoparticles to accumulate preferentially in tumor tissues expressing HER2 receptors, thereby producing enhanced contrast in MRI scans of the targeted region. This targeted approach offers immense potential for early tumor detection, monitoring of therapeutic response, and personalized treatment planning.

5.2.2. Antimicrobial Potential of Green-Synthesized IONPs against *Escherichia coli*

E. coli is a Gram-negative bacterium, a common causative agent of several severe conditions such as urinary tract infections, gastroenteritis, and bloodstream infections. The increasing prevalence of antibiotic-resistant strains—especially those producing extended-spectrum β-lactamases (ESBLs)—has driven the demand for innovative antimicrobial strategies. Several factors such as environmental pH, nanoparticle size, surface charge and concentration affect the potential of the IONPs. Smaller nanoparticles penetrate the bacterial cell more readily; their positive surface charge aids the adhesion to the cell membrane and increased ROS generation in acidic pH which can be optimised for infected wounds.

5.2.3. Anti-cancer Activity of IONPs

The cell viability test was carried on the HeLa cell lines, a well-established human cervical cancer cell line and the unique physicochemical property of the IONPs exhibit promising cytotoxic affect and can be considered for future therapeutic applications in oncology. In vitro MTT assay revealed the significant dose dependent cytotoxicity on exposure to IONPs. Functionalisation with ligands, drugs or antibodies can be employed to enhance the specificity and efficacy of the nanoparticle in targeted cancer therapy.

5.2.4. Environmental remediation using IONPs

Although experimental work was not conducted, IONPs has been proved to be potential as innovative, efficient, and sustainable remediation strategy for the removal of toxic heavy metals such as arsenic, cadmium, chromium and lead from waste water. Their magnetic property makes their removal easier minimising their secondary pollution. IONPs has great adsorption potential due to their high surface area to volume ratio, they immobilise the environmental contaminants by adsorption, reduction and precipitation mechanism. IONPs can directly be injected (in situ) or added to extracted contaminated water or soil in treatment plants or filtration system (ex situ). The potential toxicity may be reduced by cost effective and eco-friendly green approach for the large-scale synthesis of the nanoparticles.

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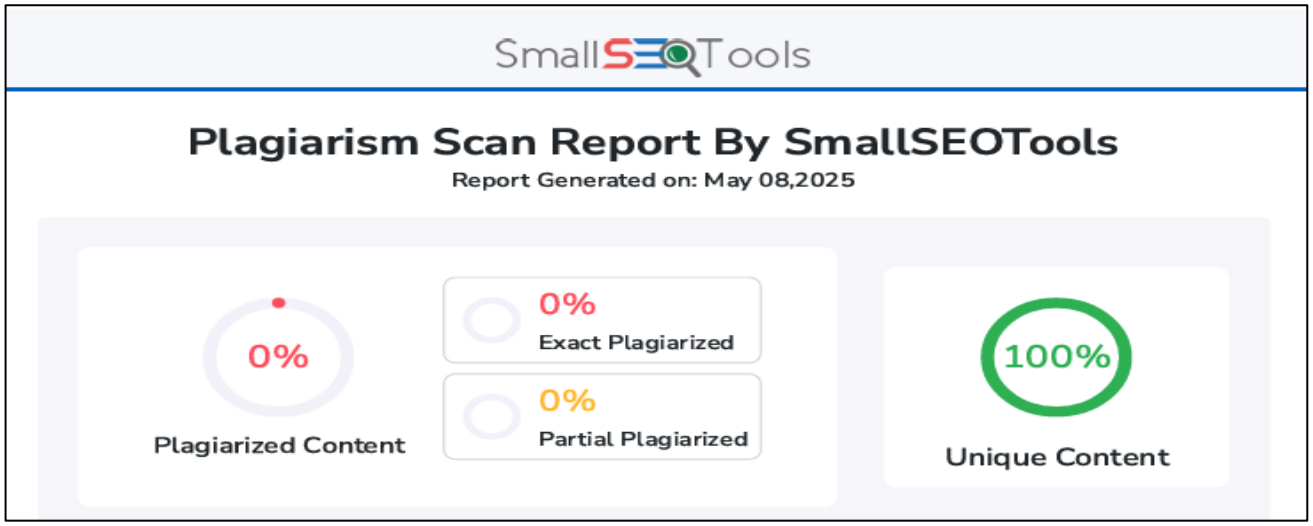

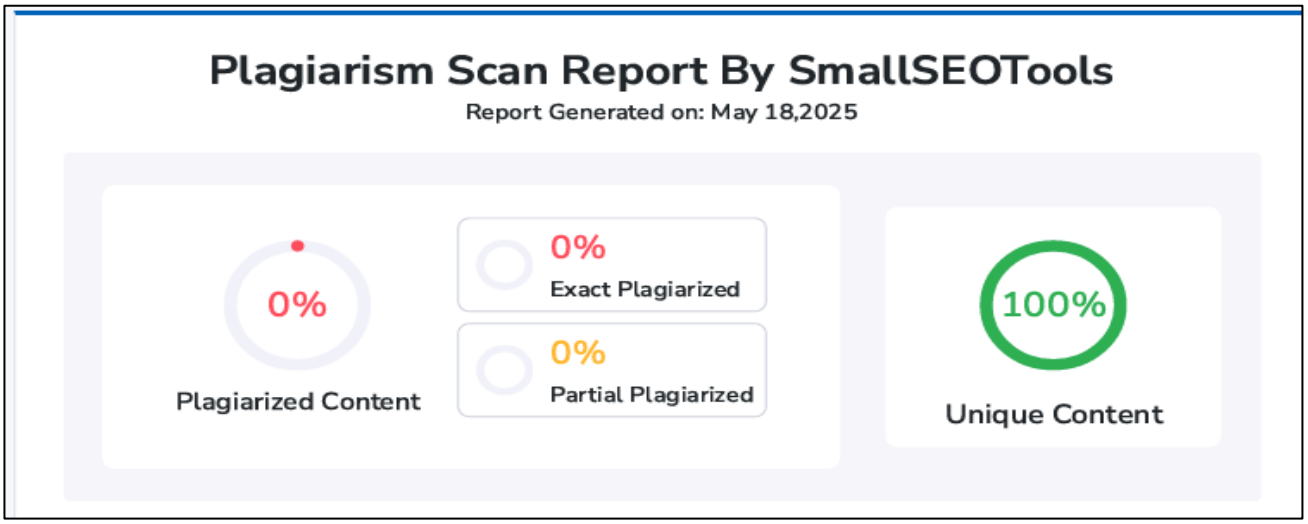

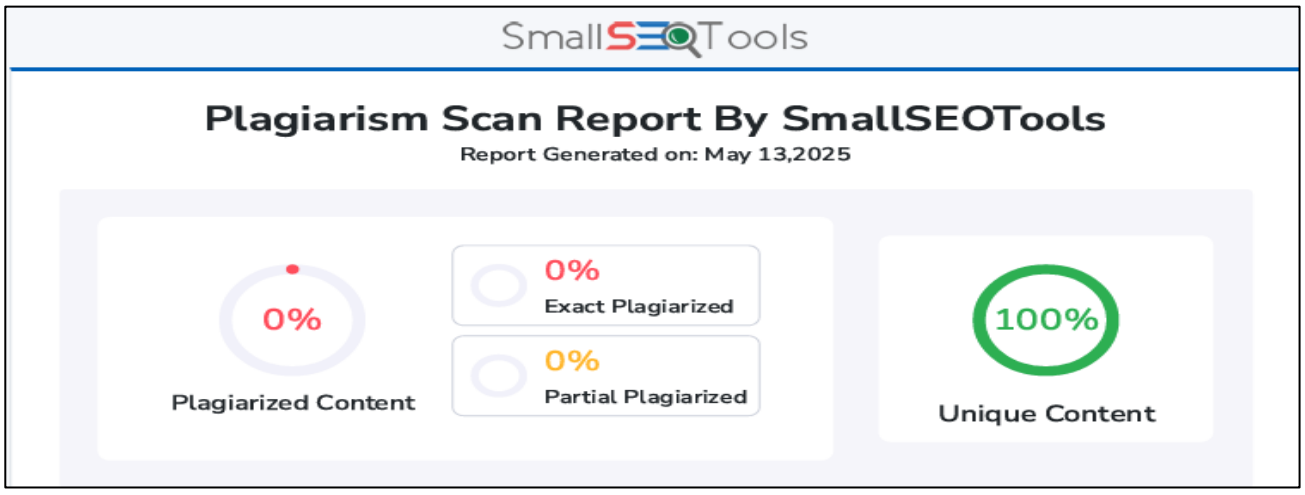

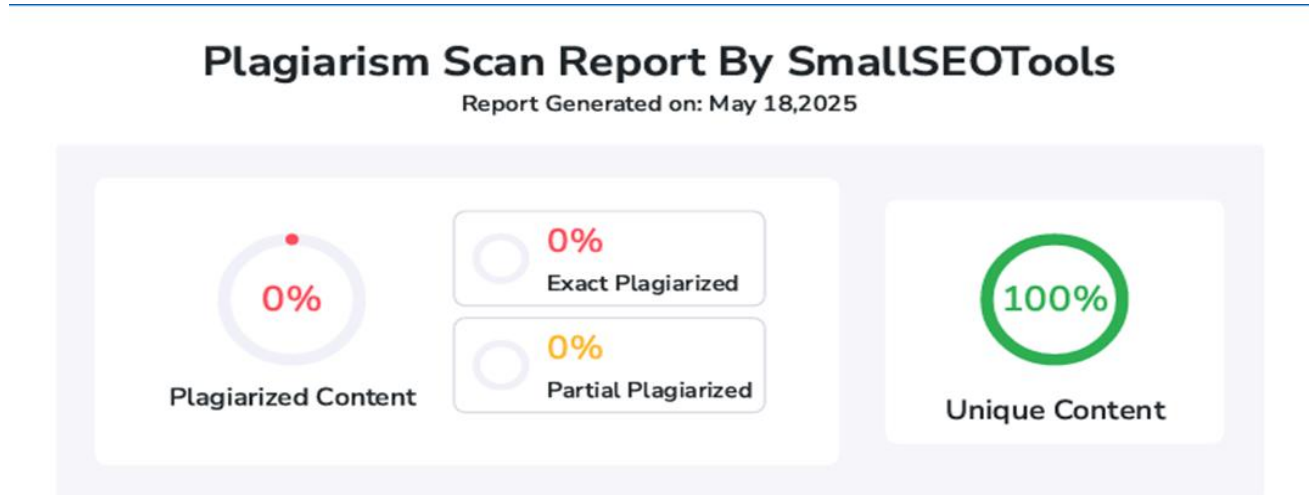
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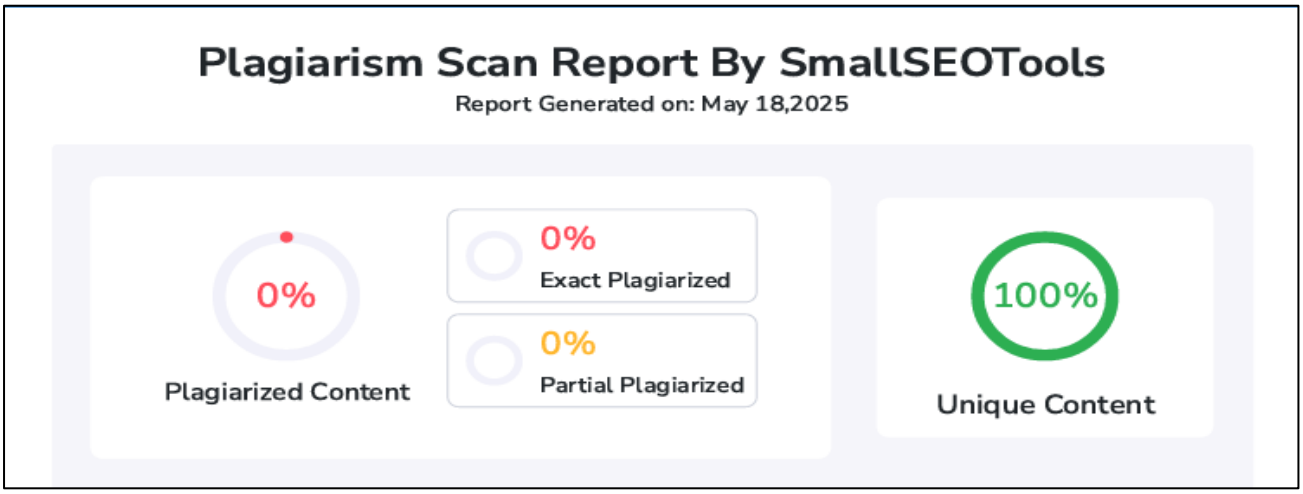
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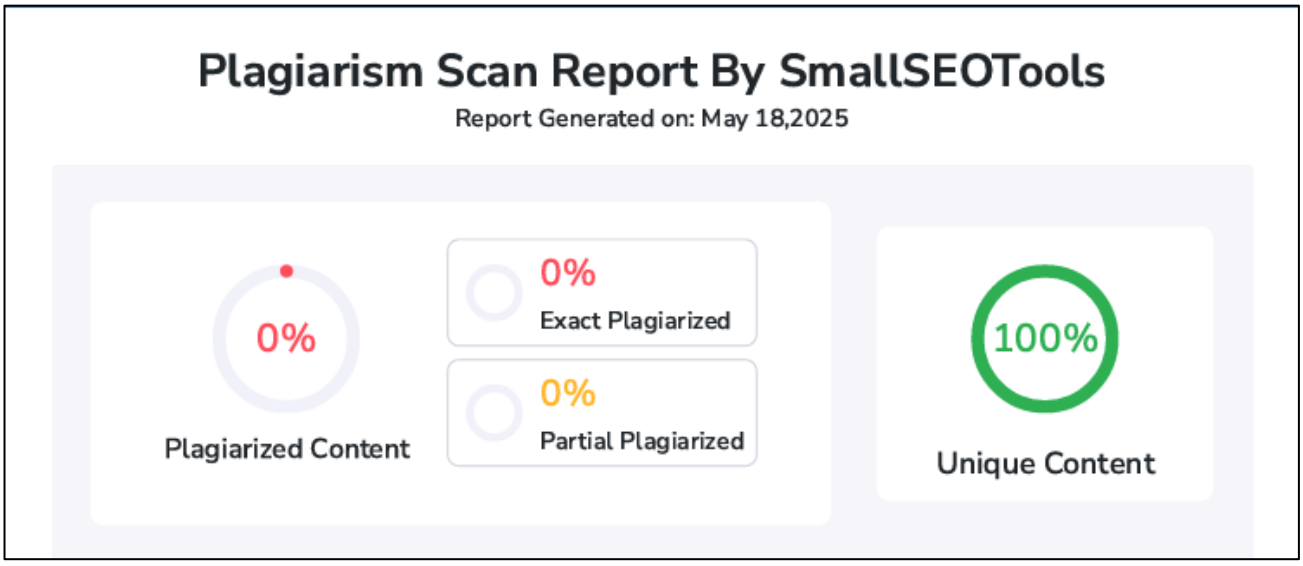
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